Preparing a Polymorphonuclear Cell Fraction by Ammonium Chloride with Potassium (ACK) Lysis of the Erythrocyte Pellet Post-Density Gradient Treatment

1. Dilute the whole blood 1:1 with PBS.
2. Layer the diluted sample over a volume of density gradient medium (e.g. Ficoll™, Lymphoprep™) equal to or greater than the initial blood volume.
3. Centrifuge for 30 minutes at 600 x g at room temperature, with the brake off. (G to RPM Converter)
4. Remove and discard the mononuclear cells which form a layer at the Ficoll:Plasma interface.
5. Carefully remove and discard the remaining plasma and density gradient medium.
6. Resuspend the red cell pellet in ACK lysis buffer : completely fill the tube with ACK and incubate on ice for 10 minutes.
7. Centrifuge for 8 minutes at 300 x g.
8. Discard the supernatant and wash the pellet in PBS + 2% FBS.
9. Resuspend the resultant polymorphonuclear cells in appropriate medium (e.g. PBS + 2% FBS).

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